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A NEW METHOD FOR MEASURING REPLACEMENT OF EPIDERMIS AND STRATUM CORNEUM IN HUMAN SKIN*

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Most of our knowledge concerning the rate of epidermal replacement has been derived from measurements of the mitotic index and from autoradiography [Katzberg, (1) Pinkus (2), Epstein and Maibach, (3)]. In addition Rothberg, Crounse and Lee (4) observed the time of first appearance and peak activity of glycine-2-C^{14} isolated from stratum corneum after systemic administration of the labelled amino-acid.

There are several disadvantages to these methods and a new technique has been devised which can be used in clinical investigation.

PRINCIPLE

The principle of the method is to measure† the transit time of epidermal cells by incorporation of locally injected C^{14} labelled amino-acids into keratin. Glycine and methionine were used and their appearance at the surface was detected in a single stripping of the stratum corneum with a cellophane tape.

MATERIALS AND METHODS

Twelve male and twelve female patients‡ all schizophrenics, whose ages ranged from thirty-four to eighty-four years took part in the experiment. There was no evidence of present or past skin disease, either in the patients or in their relatives. Treatment included psychotropic drugs, sedatives, and anticonvulsant therapy. None was receiving cytotoxic drugs or corticosteroids.

For accurate reference, sites were selected on the flexor aspect of the forearm in relation to permanent landmarks, such as nevi or freckles where possible. Glycine was injected intradermally into two sites 1½ inches apart on the right forearm and methionine similarly into two sites on the left.

It was found that injection of 0.5 μc. of glycine-1-C^{14} in 0.1 ml. saline and 0.5 μc. of L-methionine (methyl-C^{14}) in 0.05 ml. Saline gave adequate counts in a single stripping of keratinocytes. Using one inch wide transparent adhesive tape, a single stripping was obtained from each site on the fifth day and thereafter twice weekly at three and four day intervals from the fourteenth to the forty-ninth day. Patients were asked not to use any application locally nor wash their forearm in the twenty-four hours before stripping.

After stripping, a square inch of tape, cut to include the site of isotope injection at its center, was placed into a counting bottle containing 8 ml. of scintillation fluid (0.3 G/l of dimethyl POPOP, 5.0 G/l of PPO in toluene). The scintillation fluid is a satisfactory solvent but it was found essential to immerse the tape immediately after stripping as complete separation of cells from tape became increasingly difficult with time. When all the adhesive material had been dissolved, the cellophane backing was removed with forceps and the bottle placed in a Packard Tricarb Liquid Scintillation Spectrometer (Model 4312).

After the keratinocytes had separated from the tape they quickly sedimented to the bottom of the counting bottle. It was therefore important to establish whether higher counts could be obtained by a more uniform distribution of radioactivity throughout the scintillation fluid. It can be seen that lysis of the cells with hyamine caused marked quenching (Table 1) and that there was no advantage in suspending cells in Cab-O-Sil (Packard Instrument Company, Inc.), a thixotropic gel (Table 2). Further experiments also showed that neither cells nor the adhesive material on the tape caused significant quenching (Table 3).

Incorporation of the labelled amino-acids into keratinocytes was established as follows: after counting, keratinocytes and supernatant were separated by centrifugation and counted separately. It can be seen from Table 4 that the activity is almost entirely confined to the cells.

RESULTS

The count obtained from a single stripping was expressed as a percentage of the total counts obtained from all the strippings at that site. Thirty-eight sites were stripped after

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TABLE I

Counts/minute	
Scintillation fluid alone	Scintillation fluid + hyamine
510	40
280	172
233	140
264	81
335	195
228	76

The quenching effect of 0.5 ml. hyamine when added to C^{14}-labelled keratinocytes in 8 ml. scintillation fluid.

TABLE II

Counts/minute	
Scintillation fluid alone	Scintillation fluid + Cab-O-Sil
205	207
316	340
262	249
220	208
200	191
226	234

The addition of 0.32G Cab-O-Sil to each of six samples containing C^{14}-labelled keratinocytes in 8 ml. scintillation fluid.

TABLE III

Sample	Counts/min
i Background	27
ii 8 ml. scintillation fluid + 4 mμc C^{14} hexadecane	8100
iii As in ii + adhesive from 9 sq. of tape	7975
iv As in iii + unlabelled keratinocytes from similar surface area of tape	7770

To show that keratinocytes and tape adhesive did not produce significant quenching.

glycine injection and thirty-four sites stripped after methionine. The mean and standard errors of the results are shown in Figures 1 and 2. Counts on the fifth day after injection did not differ from background radioactivity (average background 21 counts/minute).

It will be seen that there was a significant increase in surface activity by day fourteen in the case of glycine and day eighteen for

TABLE IV

Glycine		Methionine	
Cells	Supernatant	Cells	Supernatant
950	30	377	35
620	34	340	22
565	31	326	28
1112	37	206	28
857	29	284	22
767	29	393	23
820	28	270	21
704	34	256	22

Separation of cells and supernatant shows radioactivity confined to keratinocytes.

methionine. Peak activity* occurred between days nineteen and twenty-four with glycine and between days twenty-three and thirty-five with methionine. There is little overlap between the standard errors and the difference between the two curves is highly significant.

There was considerable variation both in the time of appearance of peak activity and in its magnitude in different patients (Figure 3). Neither appeared to be related to the age or sex of the subjects, although we have insufficient data to exclude such a relationship.

The mean difference in the time of peak activity between upper and lower sites of injection with the same isotope in the same forearm was 2.2 days ± 1.0 S.E. for glycine (range 0–11 days) and 3.1 days ± 3.2 S.E. for methionine (range 0–7 days).

DISCUSSION

Both glycine and methionine are essential for keratin formation. Weinstein (5) and Fukuyama, Nakamura and Bernstein (6) have demonstrated their localization in the horny layer by autoradiography in pigs and rats respectively.

Rothberg et al (4) gave C^{14}-glycine orally to two patients with chronic myeloid leukemia and found just detectable amounts of radioactivity in the stratum corneum at thirteen and fourteen days, the activity rising to a maximum between the twenty-sixth and twenty-

* The time of peak activity is defined as the days during which activity lies within one standard error of the peak of the mean activity curves (Figs. 1 and 2).

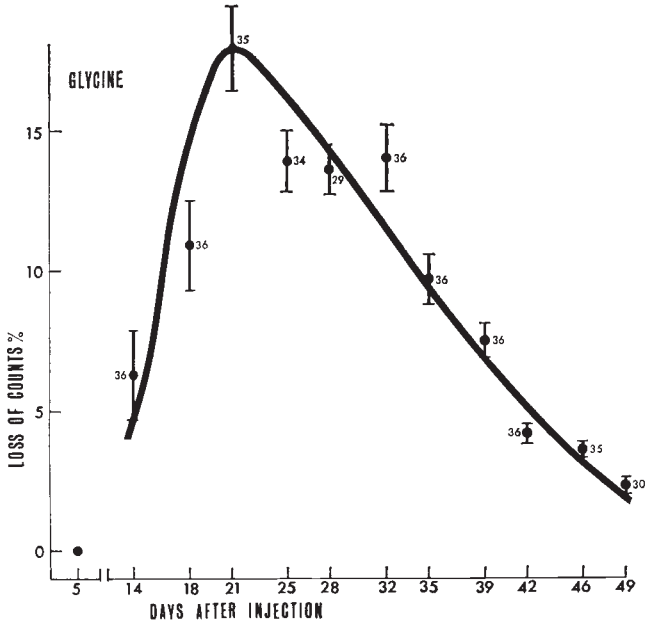

FIG. 1. Isotope obtained from single strippings at intervals after injection of 0.5 μc. of C^{14}-glycine. The count obtained from a single stripping is expressed as a percentage of the total counts obtained from all the strippings at that site. The mean and standard error are given together with the number of observations.

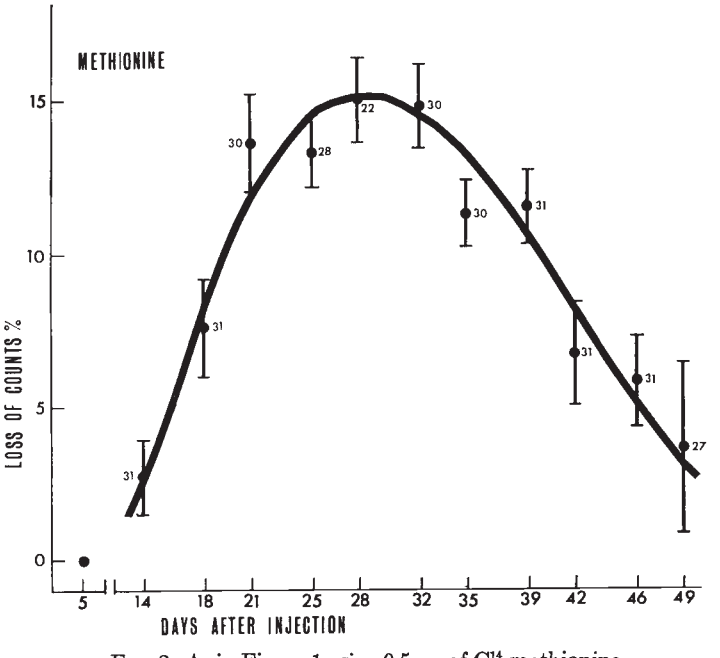

FIG. 2. As in Figure 1 using 0.5 μc. of C^{14}-methionine

eighth days. The disadvantages of this technique are the large amount of stratum corneum and the multiple chromatographic separations required to isolate glycine, the large oral dose of isotope and the removal of stratum corneum with a scalpel blade, which as the authors themselves acknowledge, is likely to stimulate mitosis. For these reasons, the method has a limited use.

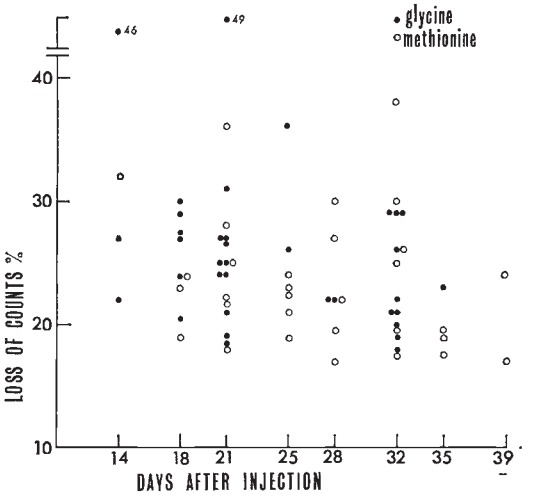

FIG. 3. Plot of peak individual peak counts expressed as a percentage of the total counts obtained from all the strippings at that site to show the wide variation in magnitude and time.

In the present technique local injection of isotope combines a low systemic dosage of radioactivity with a high local concentration. In this way it proved possible to measure radioactivity in a single stripping of horny cells. It is unlikely from the evidence of Pinkus (7) that a single stripping would greatly affect epidermal mitosis and it is improbable that a simple intradermal injection will greatly affect the rate of epidermal replacement.

Although we did not establish that glycine and methionine had been incorporated into keratin after local injection, the absence of significant activity in the supernatant and its association with the keratinocytes makes this likely.

Rothberg et al (4) proposed that the glycine moved in a band from the basal layer to the surface but their method was not designed to provide information on this point. Epstein and Maibach (3) found considerable variation in renewal time of living epidermal cells in human skin and also concluded from their autoradiographs as did Marques-Pereira and Leblond (8) using rat esophagus, that the outward movement of a basal cell is a random event.

The shape of the curves obtained in the present work are in keeping with a random upward migration if it is assumed that labelling of epidermal cells occurs during a short period of time and that there is no recycling of isotope between epidermal cells.

Regardless of the way in which cells reach the surface, the average peak loss of isotope is a measure of the time for replacement of epidermal cells. The variance of repeated measurements of replacement time in human epidermis has not been published for other methods and their reproducibility cannot therefore be compared with the present findings of 2.2 days ± 1.0 S.E. for glycine and 3.1 days ± 3.2 S.E. for methionine. One uncontrollable source of error is the variable number of cells removed at each stripping and too much reliance should not be placed on the timing of peak isotope loss from a single site.

The difference between the time of peak loss of glycine and methionine suggest different sites of incorporation of these two amino acids. Furthermore, the early sharp upstroke and the slow downstroke for glycine and the slow downstroke for methionine (Figs. 1 and 2) are precisely as would be predicted from a random movement of epidermal cells labelled with glycine in the region of the granular layer and with methionine in the region of the basal layer, but with some labelling throughout the epidermis with both. This finds support in the work of Fukuyama et al (6) who studied the localization of tritiated amino acids in newborn rat skin by autoradiography. Methionine, leucine and phenylalanine were found mainly in the basal and prickle cell layers one hour after intraperitoneal injection in contrast to glycine and histidine which were found mainly in the upper epidermis within thirty minutes of injection. Further confirmation comes from the work of Hoober and Bernstein (9) who have isolated a protein rich in tritiated glycine and histidine but not methionine, leucine or phenylalanine from newborn rat epidermis 1–1½ hours after administration of the labelled amino acids. The explanation of the different sites of incorporation of glycine and methionine may lie in the sequence with which these amino acids are incorporated into polypeptide precursors of keratin. Contrary findings in the pig (5) may be technical or due to species difference.

If, as now seems likely, these findings also hold for human epidermis, the average peak

transit time of glycine should correspond to passage from the region of the granular layer to the surface, whereas the average appearance time of the peak for methionine will correspond more closely to the time of transit through both epidermis and stratum corneum. Use of the two isotopes in this way may therefore prove useful in measuring differences between rates of replacement of stratum corneum and epidermal cells in healthy skin and in localizing a defect in abnormal skin.

SUMMARY

A new method is described for measuring the replacement of living epidermal cells and stratum corneum in human skin by observing the amount of isotope lost in single cellophane tape strippings following the intradermal injection of 0.5 μc. of glycine or methionine. Stripping commenced five days after injection and continued at three and four day intervals from the fourteenth to the forty-ninth days.

The shape of the curves of isotope loss is in keeping with a random outward passage of epidermal cells if certain assumptions can be made. Peak loss of glycine occurred at nineteen to twenty-four days and peak loss of methionine at twenty-three to thirty-five days. These findings could be explained by two separate sites of incorporation of these amino acids in the human epidermis.

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